another embodiment, wherein the mammalian subject is a human subject. In another embodiment, wherein the score is used to diagnose a neoplastic breast disease. In another embodiment, wherein the breast disease is breast cancer.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

FIG. **1** is a schematic representation of the point of care channel flow-based immunoassay detection device.

FIG. **2** is a schematic of the benchtop imaging system.

FIG. **3** shows testing for the specificity of the tested antibodies using western blotting with five antigens.

FIG. **4** shows optimization of capture and detector antibody concentrations for developing standard curves using enzyme linked immunosorbent assay (ELISA).

FIG. **5** are standard curves using ELISA.

FIG. **6** is a schematic of the sandwich immunoassay format used in protein microarrays.

FIG. **7** shows optimization of capture and detector antibody concentrations for developing standard curves using the antibody microarray immunoassay.

FIG. **8** is visualization of standard curves using the antibody microarrays.

FIG. **9** is a comparison of standard curves obtained in a single plex and a multiplex format. using the antibody microarrays, with phosphate buffered saline (PBS) and Serum as the medium.

FIG. **10** is a comparison of standard curves obtained using antibody microarrays with the ELISA.

FIG. **11** shows multiplexed assays on protein microarrays.

FIG. **12** shows the experimental layout of the breast cancer sample pilot study.

FIG. **13** shows the pilot studies with 30 breast cancer patient samples.

FIG. **14** shows the experimental layout of the breast cancer study.

FIG. **15** shows the measurement of biomarkers in breast cancer patient sera.

FIG. **16** is the standard curves for biomarkers in multiplex format.

FIG. **17** shows clustering and linearization of the four dimensional data obtained using the multiplex assay and principle component analysis.

FIG. **18** shows classification error estimate using linear discriminant analysis; H=Her-2; M=MMP-2; O=OPN; C=CA 15-3.

FIG. **19** is a schematic representation of a lateral flow test strip.

FIG. **20** shows fluorescence images of the lateral flow assay (LFA).

FIG. **21** shows fluorescence images of the sandwich LFA.

FIG. **22** shows the results of the proof of principle on microarray channels.

FIG. **23** shows standard curves using Quantum Dots on microarray channels.

FIG. **24** shows the multiplex assay using Quantum Dots on microarray channels.

FIG. **25** shows the results of troubleshooting the Quantum Dot assay on microarray channels.

FIG. **26** is the standard curves obtained using microarray channels in 15 min.

FIG. **27** is a demonstration of multiplexed immunoassays on microarray flow channels.

FIG. **28** is a determination of the best combination of assay speed and sensitivity for the microarray flow channels.

FIG. **29** shows biomarker concentration in patient serum samples.

FIG. **30** shows imaging system standard curves.

FIG. **31** is a comparison of the imaging system and a photomultiplier tube (PMT).

DETAILED DESCRIPTION

Briefly, and as described in more detail below, described herein is a channel flow based immunoassay detection device for determining the presence and/or concentration of a plurality of biomarkers in a sample. Also disclosed are methods of using the device, and arrays of capture molecules, e.g., antibodies, for use with the device. In one embodiment, the device is used to detect a plurality of biomarkers related to breast cancer. Described herein are methods of scoring a sample using data associated with the breast cancer biomarkers.

Advantages of this approach are numerous. The device provides the ability to perform multiplexed analysis of multiple biomarkers in a format that is simple to use, amenable to automation, and in a small, rugged format. The device has been developed in a point of care (POC) format, allowing for rapid diagnostic assay, and facilitating faster therapeutic decisions and possible increased patient survival rates.

The device can be used to diagnose and prescribe treatment for a wide variety of medical conditions, especially cancers, heart diseases, respiratory diseases, and microbial infections. In one embodiment, the device is used to diagnose breast cancer.

Also disclosed is a multiplexed immunoassay to detect a set of biomarkers associated with breast cancer. The immunoassay can accurately detect a panel of two, three, four, five, six, seven, or eight biomarkers from the sera of breast cancer patients and distinguish between control, early stage, and metastatic breast cancer populations. The immunoassay was shown to predict the stage of unknown sample. The assay can be used can be used along with mammography for result validation and in between annual mammograms to diagnose rapidly-growing tumors. The advantage of the multiplex assay is the ability to determine the levels of these markers simultaneously, thus reducing time, effort, overall volume of reagent and patient sample. Such a panel can offer a complete range of tests such as diagnosis, prognosis, treatment options and treatment monitoring in a single assay, providing additional information enabling rapid diagnosis and improved patient survival rates.

DEFINITIONS

Terms used in the claims and specification are defined as set forth below unless otherwise specified.

A "capture molecule" is a molecule that is immobilized on a surface. The capture molecule generally, but not necessarily, binds to a target or target molecule, e.g., a biomarker. The capture molecule is typically an antibody, a peptide, or a protein. In the case of a solid-phase immunoassay, the capture molecule is immobilized on the surface of a solid support and is an antibody specific to the target, an antigen or epitope, to be detected. The capture molecule can be labeled, e.g., a fluorescently labeled antibody or protein. The capture molecule can or can not be capable of binding to just the target. Capture molecules can include e.g., RNA, DNA, peptides,